

Appl. No. 10/506,651

PATENT

Amdt. dated October 21, 2004

Reply to Notification of Missing Requirements of October 14, 2004

submit herewith the required paper copy and computer readable copy of the Sequence Listing.

Please amend the specification as follows.

Amendments to the Specification begin on page 3 of this paper.

Remarks begin on page 6 of this paper.

Amendments to the Specification:

Please replace paragraph [19] beginning at page 5, line 27, with the following:

--[19] Figure 2 shows the cleavage sites in the gp160 (env 89.6) protein which delimit both the truncated and untruncated forms of gp41 (DNA SEQ. = SEQ ID NO:5; AA SEQ. = SEQ ID NO:6).--

Please replace paragraph [25] beginning at page 6, line 29, with the following:

--[25] The term "gp160" refers to the human immunodeficiency virus-1 (HIV) gene encoding the HIV envelope glycoprotein illustrated by example in ~~SEQ ID:1~~ SEQ ID NO:1. gp160 comprises two coding regions, one encoding the 120kDa (gp120) of the envelope glycoprotein and the other encoding the 41kDa subunit (gp41) which includes a transmembrane region and a cytoplasmic tail. In the context of this invention, the term "gp160" also refers to a truncated version of gp160 alternatively termed "gp140". This truncated version lacks the transmembrane subunit and the cytoplasmic tail which is defined as the 3' end of the gp160 gene sequence, beginning within 5 amino acids either side of residue 684 as noted in SEQ ID NO:3.--

Please replace paragraph [81] beginning at page 21, line 26, with the following:

--[81] Exemplary linkers of the present invention include sequences selected from the group of formulas:
(GlySer)_n (SEQ ID NO:15), (Gly₃Ser)_n (SEQ ID NO:16), (Gly₄Ser)_n (SEQ ID NO:17),
(Gly₅Ser)_n (SEQ ID NO:18), (Gly_nSer)_n (SEQ ID NOS:19-28) or (AlaGlySer)_n (SEQ ID NO:29)
where n can take a value within the range 3 to 12. Additional examples of preferred linkers are set out in SEQ ID NOS:10 through 14.--

Please replace paragraph [85] beginning at page 22, line 29, with the following:

--[85] Regardless of which form or variant of the gp160 gene is used, a fusion protein between gp120 and gp41 joined by a flexible linker can be created by identical methodology known in the art (see Maniatis, Fritsch and Sambrook, *Molecular Cloning: A Laboratory Manual*, 2nd Ed. (1989); *DNA Cloning: A Practical Approach*, Volumes I and II (D. N. Glover ed. 1985); *Current Protocols in Molecular Biology* (Ausubel et al., eds., 1994)). For example, in a preferred embodiment of the invention, the codons for two amino acid residues (KR to ID) are mutated in the region encoding the proteolytic cleavage site between gp120 and gp41. These mutations create two new restriction sites, EcoRI and EcoRV, which allows for the incorporation a polypeptide linker having ends of (~~GGSGG~~) GGSGG (SEQ ID NO:33). Examples of the complete primary sequence of fusion proteins constructed in this manner are set out in SEQ ID NOS:7 and 8.--

Please replace paragraph [90] beginning at page 24, line 1, with the following:

--[90] Nucleotide sequences encoding gp41 are similarly common and can be recovered from any HIV isolate using for example labeled probes derived from ~~SEQ IDs 1~~ SEQ ID NOS:1 or 3. gp41 coding sequences can also be isolated from known gp160 and gp140 sequences by molecular biological techniques known in the art, such as those described supra. In this latter context, gp41 coding subunits used to construct the fusion proteins of this invention can be either the full-length form having the transmembrane subunit, or the truncated form derived from the gp140 variant which lacks the coding sequence for the transmembrane subunit.--

Please replace paragraph [151] beginning at page 42, line 11, with the following:

--[151] Stable fusion proteins of gp120 and gp41 joined by flexible linkers were created using the envelope glycoprotein from the primary R5X4 HIV-1 isolate 89.6 as starting material. Two

amino acid residues of the post translational cleavage site REKR (SEQ ID NO:30), were mutated by PCR changing the sequence of the site to REID (SEQ ID NO:31). Two new restriction sites, EcoRI and EcoRV, were introduced into the sequence. Introduction of the restriction sites created a short fragment (EFIS; SEQ ID NO:32) following the mutated cleavage site. Flexible linkers were introduced into the middle of this sequence by PCR. Three different fusion proteins where gp120 and gp41 are joined by fragments of different total lengths 4 (SEQ ID NO:9), 15 (SEQ ID NO:10) or 26 (SEQ ID NO:11) amino acid residues were developed. Using the same technique, a stop codon was introduced at position 668 of the *env* protein sequence (GenBank accession numbers U39362, AAA81043) and three additional amino acids (KLV) added at the very end of the linker proteins. The stop codons result in proteins that are truncated N-terminal to the transmembrane domain of gp41. Since the fusion proteins do not contain the transmembrane domain and cytoplasmic tail of gp41, they are secreted in the medium of the expressing cells. Thus, three different fusion proteins, designated gp140-4, gp140-15 and gp140-26 were developed.--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1-22, at the end of the application.